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Full Length Research Paper

Normal bacterial flora from idmi (*Gazella gazella*) and reem gazelles (*Gazella subgutturosa marica*) in Saudi Arabia

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Nasal, buccal and rectal swabs were collected from 24 adults and 25 newborn clinically healthy reem (*Gazella subgutturosa marica*) and idmi (*Gazella gazella*) gazelles from the breeding pens of the King Khalid Wildlife Research Center (KKWRC), Riyadh, Saudi Arabia. Swabs were cultured on bacteriological media and organisms isolated were identified using the appropriate analytical profile index (API) identification system. 253 bacterial and 5 fungal isolates were reported from idmi and reem under investigation. Of the bacteria isolated, 136 (53.8%) were isolated from idmi gazelles whereas 117 (46.2%) were isolated from reem gazelles. 187 (73.9%) of the isolates were Gram positive while 66 (26.1%) were Gram negative. Among the significant Gram negative organisms isolated was *Fusobacterium necrophorum* which was isolated from both species under investigation. The pathogenic Gram positive organism, *Arcanobacterium pyogenes* was also isolated from reem and idmi gazelles at the centre. *Listeria grayi* was isolated only from idmi gazelles whereas unidentified species of *Nocardia* was isolated from reem gazelles. The only fungal species isolated was *Candida albicans* which was isolated from the rectal swabs of idmi gazelles.

Key words: *Gazella gazella, Gazella subgutturosa marica*, bacteria, yeast, normal flora.

INTRODUCTION

The number of mountain gazelle or idmi (*Gazella gazella*) and sand gazelle or reem (*Gazella subgutturosa marica*) are in continuous decline due to overhunting and habitat fragmentation (Thouless et al., 1991). King Khalid Wildlife Research Centre has established a captive breeding program for these two species of gazelles. Sizeable herds of reem and idmi gazelles were kept in a large enclosure of 600 ha (Kichenside and Lindsay, 1997). Gazelles were caught and translocated into the new breeding pens each measuring 100 × 50 m. During the process of moving gazelles from the big enclosure to the breeding pens, tuberculosis was discovered in gazelles and the Arabian oryx (*Oryx leucoryx*) (Rietkerk et al., 1993). Gazelles were exposed to the infection as they tend to share feeding sites with the Arabian oryx and other animal species, which are highly susceptible to infection with tuberculosis. Among, which is a group of fallow deer (*Dama dama*) has been proven later as the main source of the infection. A strict eradication program

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was followed after that and few years later all gazelles at the Centre were declared free of tuberculosis. The idmi population at the Centre was used for the first reintroduction in Ibex Reserve (Dunham et al., 1993). The reem gazelles from the Centre were also used for the successful reintroduction program in the Uruq Bani Ma'arid Protected Area at the western edge of the Empty Quarter (Wacher and Kichenside, 1998).

Molecular evidence was shown by Wacher et al. (2011) that the two subtaxa of *G. subgutturosa* that is *G. subgutturosa marica* and *G. subguutosa subgutturosa* may have evolved independently as previously suggested by Hammond et al. (2001). On the other hand, Idmi or mountain gazelles have shown monophyletic genetic lineages within the *G. gazella* complex one northern clade or group and another clade comprising all other *G. gazella* from the Arabian Peninsula (Wronski et al., 2010). In the present study, bacteria and fungi normally inhabiting the mouth, rectum and nose were evaluated from adult and newborn reem and idmi gazelles held at King Khalid Wildlife Research Centre.

MATERIALS AND METHODS

Samples were collected from 24 and 25 adult and newborn clinically healthy reem and idmi gazelles, respectively. From each individual swabs from the mouth, nose and rectum were collected on a transport medium (Aimes, USA). Gazelles in the breeding pens of the King Khalid Wildlife Research Centre were caught using the routine method of capture designed to handle gazelles at the Centre (Kichenside and Lindsay, 1997). Newborn gazelles were caught when they were just born or when they were one day old as they are normally handled for weighing and ear tagging. In the laboratory, swabs were streaked into different culture media (SPML, Riyadh, Saudi Arabia) such as sheep blood agar (SBA), MacConkey agar (MA); Neomycin anaerobe blood agar (NABA) and Columbia PNBA agar (CAN). In addition the swabs were cultured into Sabouraud-dextrose agar (SDA) for fungal isolation. SBA is a non selective medium and used or primary isolation of bacteria. MA, NABA and CAN were selective media for isolating Gram negative, anaerobic and Gram positive organisms respectively. Inoculated culture media were incubated at 37°C for 18 to 24 h. Fugal culture media were incubated at 30° C for one week. Cultured organisms were recognized from their colonial morphology and subcultured in order to obtain pure cultures for identification purposes.

Tentative identification was established according to the colonial and cellular morphology, Gram stain, spore formation, aerobic and anaerobic growth, catalase and oxidase reactions (Cowan and Steel, 1974). Specific identification was achieved using the API system for identification [analytical profile index (API), Montaliue, Vercieu, France]. Seven types of API systems were used:

i) API Coryne for identification of Corynebacteria and Coryneform bacteria.
ii) API Staph for identification of Staphylococci and Micrococci.
iii) API 20 Strep for identification of Streptococci and related bacteria.
iv) API 20 A for identification of anaerobes.
v) API 20 C AUX for identification of yeast.
vi) API 20 E for identification of Gram-negative organisms.
vii) API 50 CHB for identification of *Bacilli*.

RESULTS

253 bacterial and 5 fungal isolates have been reported from idmi and reem gazelles under investigation. A total of 39 bacterial species have been isolated from reem and idmi gazelles at King Khalid Wildlife Research Centre (KKWRC). Only one fungus (*Candida albicans*) has been isolated during the present study. The majority of bacterial isolates were from the lower digestive tract. 185 isolates were Gram positive organisms, whereas, 68 isolates were Gram negative organisms. Of the bacterial isolates, 136 (53.8%) were isolated from idmi gazelles (Tables 1 and 2), whereas 117 (46.2%) were isolated from reem gazelles (Tables 3 and 4). A total of 186 (73.5%) of the isolates were Gram positive while 67
(26.5%) were Gram negative. In idmi gazelles, 80 bacterial isolates were from adults (Table 1) while 56 isolates were from newborns (Table 2). Of the 136 idmi isolates, 102 were Gram positive and 34 isolates wee Gram negative. In reem gazelles, 60 isolates were from adults (Table 3) and 57 isolates were from newborns (Table 4). Of these 84 isolates were Gram positive and 33 were Gram negative. 49 isolates were *Staphylococcus* species, 15 from idmi and 34 from reem.

Most of the *staphylococcus* isolates were from the mouth. *Streptococcus* species have been isolated on 38 occasions, 24 isolates were from idmi and 14 were from reem gazelles.

DISCUSSION

In the present study, bacteria and fungi of the upper respiratory and lower digestive tracts of reem and idmi gazelles have been identified. Organisms involved have been identified to the species level using the standard API (analytical profile index) identification system. The frequency of Gram positive organisms isolated from gazelles is much greater than Gram negative organisms isolated from each individual. This indicates that gazelles species under investigation are more susceptible to Gram positive organisms or they may possibly harbour more Gram positive organisms. Despite the low number of Gram negative bacteria isolated, species isolated are known to be pathogenic and they have been associated with disease. Among Gram negative organisms was *Fusobacterium necrophorum* which is a common cause of infections associated with production of pus. It is involved in dental and periodontal infections, ulcerative gingivitis, liver abscess and cerebral abscess (Collee et al., 1989). This anaerobic organism was found to be the principal isolate in any pyogenic infection in gazelles at KKWRC (unpublished data). Idmi seems to be more susceptible to the infection by this organism compared to reem. It is interesting to isolate this organism only from the lower digestive tract as it is usually associated with lung infections. It is likely that the organisms would enter

Table 1. Organisms isolated from Adult Idmi gazelles (*Gazella gazelle*) n = 12.

Organism	Source			
Gram-negative bacteria	**Mouth**	**Nose**	**Rectum**	**Total**
Acinetobater calco.lowffii	3	1	0	4
Bacteroides ovatus	0	0	1	1
E. coli 1	0	0	11	11
E. coli 1 (haemolytic)	0	0	1	1
*Fuosobacterium necrophrum**	0	0	1	1
Proteus mirabilis	0	0	1	1
Pseudomonas paucimobilis	2	2	0	4
Gram-positive bacteria				
Aerococcus viridans 2	2	0	0	2
Aerococcus viridans 3	4	1	0	5
Bacillus cerreus 1	1	7	0	8
Bacillus licheniformis	2	4	2	8
Bacillus subtilis	0	1	0	1
*Clostridium butyricum**	0	0	1	1
Arcanobacterium pyogenes	1	0	0	1
Enterobacter aerogenes	0	2	0	2
Enterococcus feacalis	1	0	3	4
Enterococcus galinarum	0	0	2	2
Gemella haemolysans	3	0	0	3
Listeria grayi	0	2	0	2
Micrococcus leuteus	1	0	0	1
Micrococcus lylae	2	1	0	3
Staphylococcus lugdunensis	2	0	0	2
Staphylococcus sciuri	2	0	2	4
Streptococcus acidominimus	3	0	0	3
Streptococcus cermoris	1	0	0	1
Streptococcus morbillorum	0	2	0	2
Streptococcus sangius	1	0	1	2
Yeasts				
Candida albicans	0	0	5	5
Total	31	23	31	85

*Anaerobic bacteria.

Table 2. Organisms isolated from newborn Idmi gazelles (*Gazella gazelle*) n = 12.

Organism	Source			
Gram-negative bacteria	**Mouth**	**Nose**	**Rectum**	**Total**
Acinetobater calco.lowffii	0	0	1	1
E. coli 1	0	0	6	6
*Fuosobacterium necrophrum**	0	0	3	3
Pseudomonas paucimobilis	1	0	0	1
Gram-positive bacteria				
Bacillus licheniformis	0	4	1	5
Bacillus subtilis	0	1	0	1
*Clostridium butyricum**	0	0	1	1

Table 2. Contd

*Clostridium perfrings**	0	0	2	2
Arcanobacterium pyogenes	3	0	0	3
Gardnerella vaginalis	3	0	0	3
*Lactobacillus acidophilis**	0	0	1	1
Nocardia sp.	0	2	2	4
Staphylococcus cohnii ssp cohnii	1	0	0	1
Staphylococcus lugdunensis	2	0	0	2
Staphylococcus sciuri	1	3	2	6
Streptococcus acidominimus	5	2	2	9
Streptococcus bovis	0	0	2	2
Streptococcus equinus	3	0	0	3
Streptococcus lactis	2	0	0	2
Total	21	12	23	56

*Anaerobic bacteria.

Table 3. Organisms isolated from adult Reem gazelles (*Gazelle subgutturosa marica*) n = 12.

Organism	**Source**			
Gram-negative bacteria	**Mouth**	**Nose**	**Rectum**	**Total**
Acinetobater calco.lowffii	1	0	0	1
E. coli 1	0	0	8	8
E. coli 1 (haemolytic)	0	0	3	3
Enterobacter aerogenes	0	0	3	3
*Fuosobacterium necrophrum**	0	0	3	3
Gram-positive bacteria				
Bacillus cerreus 1	0	4	0	4
Bacillus licheniformis	0	4	0	4
Bacillus subtilis	0	1	0	1
Corynebacterium aquaticum	1	0	0	1
Corynebacterium jekium	1	0	0	1
Arcanobacterium pyogenes	4	0	0	4
Corynebacterium xerosis	1	0	0	1
Micrococcus lylae	3	0	0	3
Nocardia sp.	0	0	1	1
Staphylococcus lugdunensis	2	0	0	2
Staphylococcus sciuri	4	3	0	7
Staphylococcus simulans	2	1	0	3
Streptococcus acidominimus	6	0	0	6
Streptococcus morbillorum	3	0	0	3
Streptococcus sangius	1	0	0	1
Total	29	13	18	60

*Anaerobic bacteria.

the body through the lower digestive tract and then migrate upwards to the other visceral organs.

Members of the genus *Enterobacter* especially *E. cloacae* and *E. aerogenes* are frequently isolated in infections associated with hospitalized and debilitated patients (Gaston, 1988). More recently, *E. aerogenes* has emerged as an important nosocomial pathogen because of its high level of resistance to antibiotics (Mellencamp,

Table 4. Organisms isolated from newborn Reem gazelles (*Gazella subgutturosa marica*) n = 13.

Organism	Source			Total
Gram-negative bacteria	**Mouth**	**Nose**	**Rectum**	
Acinetobater calco.lowffii	4	0	0	4
Enterobacter aerogenes	0	1	1	2
Pseudomonas paucimobilis	5	4	0	9
Gram-positive bacteria				
Bacillus cerreus 1	0	4	0	4
Bacillus licheniformis	1	6	0	7
Nocardia sp.	1	1	3	5
Staphylococcus lugdunensis	2	3	1	6
Staphylococcus sciuri	11	1	3	15
Staphylococcus simulans	0	0	1	1
Streptococcus acidominimus	4	0	0	4
Total	28	20	9	57

et al.,1990). This organism occurs in water, soil and sewage and it contributes to causing coliform mastitis in cattle, uterine infections in mares and occasionally part of the metritis-agalactia syndrome in sows (Quinn et al., 1994). *E. aerogenes* has been isolated as a normal flora only from reem gazelles both in adults and newborns and it appaeared that idmi gazelles environemnet is unsuitable for the growth of this organism. *Acinetobacter lwoffi* is related to the family Neisseriaceae which includes members of the genera *Moraxella* and *Kingella* in addition. *A. lwoffi* is usually free living saprophyte of soil, water and sewage and has been isolated from clini-cal specimens of human and animal origins (Baumann, 1968). This organism was originally considered as nonpathogenic, but it is newly recognized as playing a significant role in the colonization and infection in immunocompromised patients in intensive care units (Bergogne-Berezin and Towner, 1996). *A. lwoffi* is resistant to antibiotic and it is likely that this organism will be of increasing epidemiological importance in future (Crowe et al., 1995).

It has been reported as a normal flora of humans and animals and it can cause infections in immune-com-promised patients and Quinn et al. (1994) have isolated it from the blood of a sick dog. It is probably that this organism is available in soil and water and likely to conta-minate gazelles' water. Among Gram positive organisms isolated from gazelles, *Arcanobacterium pyogenes* (formerly *Actinomyces pyogenes*), *Corynebacterium* and *Clostridium* spp. are the most significant isolates. Actinomycetes are common commensals of the oral mucosa of animals. *A. pyogenes* is a ubiquitous opportunistic pathogen commonly isolated from gazelles at the centre that die as a result of suppurative granulumatous pneumonia or localized abscesses. It may be involved in a variety of pleurisy and mastitis (Timoney et al., 1988). On many occasions this organism has been associated with lung, liver, jaw and deep seated abscesses. In most instances it was found to be associated with the anaerobic Gram negative organism *F. necrophorum* (Quinn et al., 1994). Isolation of *A. pyogenes* as a normal flora from the mouth of gazelles could be explained by the fact that this organism is available in soil and it contaminates gazelles' food and water. The organism could cause disease in immuno-compromised animals. It is interesting to note that this organism has been more frequently isolated from idmi neonates and from adult reem gazelles. This may indicate that the immune system of neonate reem is well developed compared to neonate idmi gazelles which appears to be less immunocompetant.

Members of the genus *Clostridium* are anaerobic, large, straight or slightly curved Gram-positive rods. Most members of the genus are nonpathogenic and are commonly found in soil and intestinal tracts of man and animals (Jones and Hunt, 1983). *Clostridium perfringens* (the causative agent of enterotoxaemia) has been isolated only from the rectum of two reem neonates. Interestingly, the affected gazelles did not show signs of disease. It is possible that the strain involved is less virulent because the virulence of the bacterial strain is largely dependent on its ability to produce different toxins (Rood and Cole, 1991). This pathogenic bacterium has been isolated from gazelles during an outbreak at Prince Mohammed Al Sudairy Gazelle Research Centre at Qassim, Saudi Arabia. During this outbreak more than 80 reem gazelle have died and the main organisms isolated from dead gazelles were *C. perfringens* and *Clostridium subterminale*. Isolating *C. perfringens* from a neonate reem gazelle is a significant finding. It indicates that neonates could play a vital role in the epidemiology of clostridial infections in gazelles especially in immunocompromised gazelles.

The most frequently isolated organism was *Staphylococcus scuiri* and it has been isolated on 32 occasions. It has been isolated from healthy and sick domestic animals including cats, dogs, cattle, sheep, goat, horse and pigs (Isabel et al., 2000). Although it is rarely associated with colonization or infection in humans, it has been occasionally isolated from human clinical samples (Adegoke, 1986). This organism has been isolated from all organs investigated and from both species of gazelles. It is known as a causative agent of pyogenic infections in animals and man. During this investigation it has been isolated from healthy individuals. The majority of isolations were from neonate reem gazelles. It is probably that this organism is naturally occurring in healthy gazelles and not causing any disease in the gazelle species under investigation.

Members of the genus *Bacillus* are generally nonpathogenic with the exception of the *Bacillus anthracis* which is known to cause serious disease in man and animals. Other members of the genus *Bacillus* have been reported to be pathogenic under certain conditions such as *B. cereus* which has been associated with food poisoning in humans and with abortion or perinatal mortality in sheep and cattle. *B. cereus* has been isolated in many occasions from both reem and idmi gazelles. It is surprising that it was not associated with disease and the possible explanation for that is gazelles may have developed some immunity against infection with this organism. *Bacillus subtilis* and *Bacillus licheniformis* which have also been isolated during this study are not known as pathogenic in man or animals (Knisely, 1996). Although *B. licheniformis* was reported to cause abortion in sheep and cattle (Turnbull and Kramer, 1995); in this study it has been isolated from gazelles as normal flora and it has not been associated with disease. Six species of *Streptococcus* species have been isolated from both reem and idmi gazelles as normal flora. None of which appeared to be associated with disease with the exception of *S. bovis* which has been associated with sporadic cases of human endocarditis and bovine mastitis (Hardie, 1986; McDonald and McDonald, 1976). During the present study, it has been only isolated from the rectum of idmi gazelle neonates and it was not found to be associated with pathology.

Members of the genus *Listeria* are generally non pathogenic with the exception of *Listeria moncytogenes* (Collee et al., 1989). *L. moncytogenes* is responsible of causing the neurological disease listeriosis or circling disease in man and animals. The organism owes its name from to the fact that its infection in rabbits causes a monocytosis (Collee et al., 1989). *Listeria grayiis* which has been isolated only from adult idmi gazelles are rarely isolated and considered to be non pathogenic as other members of the genus (Quinn et al., 1994). Isolating *Nocardia* sp. from gazelle neonates is a significant one. It was difficult to identify the species involved and it is certainly not *Nocardia farcinica*. *N. farcinica* which may be identical to *Nocardia asteriodes* is reported as the cause of a disease in cattle known as bovine farcy (Jones and Hunt, 1983). It is recommended to characterise the specific identity of *Nocardia* isolated during the present study. *Candida albicans* has been isolated on 5 occasions from the rectum of adult idmi gazelle. This finding is very important and it could indicate that the reem gazelle are possibly resistant to the infection with *C. albicans*. The organism has been isolated before from ostrich chicks at the centre. The possible source of the organism could originate from birds in the area.

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